Quantitative selection of DNA aptamers through microfluidic selection and high-throughput sequencing.

Journal: Proc Natl Acad Sci U S A

Publication Year: 2010

Authors: Minseon Cho, Yi Xiao, Jeff Nie, Ron Stewart, Andrew T Csordas, Seung Soo Oh, James A Thomson, H Tom Soh

PubMed link: 20705898

Funding Grants: Training Program in Stem Cell Biology and Engineering

Public Summary:
We describe the integration of microfluidic selection with high-throughput DNA sequencing technology for rapid and efficient discovery of nucleic acid aptamers. Aptamers are small DNA molecules that bind protein targets. They might be useful in stem cell research because they could be used to sort out specific cell types. The Quantitative Selection of Aptamers through Sequencing method tracks the copy number and enrichment-fold of more than 10 million individual sequences through multiple selection rounds, enabling the identification of high-affinity aptamers without the need for the pool to fully converge to a small number of sequences. Importantly, this method allows the discrimination of sequences that arise from experimental biases rather than true high-affinity target binding. As a demonstration, we have identified aptamers that specifically bind to PDGF-BB protein with K(d) < 3 nM within 3 rounds. Furthermore, we show that the aptamers identified by Quantitative Selection of Aptamers through Sequencing have approximately 3-8-fold higher affinity and approximately 2-4-fold higher specificity relative to those discovered through conventional cloning methods. Given that many biocombinatorial libraries are encoded with nucleic acids, we extrapolate that our method may be extended to other types of libraries for a range of molecular functions. This method makes it easier to obtain reagents that can be used to sort out specific stem cell derivatives.

Scientific Abstract:
We describe the integration of microfluidic selection with high-throughput DNA sequencing technology for rapid and efficient discovery of nucleic acid aptamers. The Quantitative Selection of Aptamers through Sequencing method tracks the copy number and enrichment-fold of more than 10 million individual sequences through multiple selection rounds, enabling the identification of high-affinity aptamers without the need for the pool to fully converge to a small number of sequences. Importantly, this method allows the discrimination of sequences that arise from experimental biases rather than true high-affinity target binding. As a demonstration, we have identified aptamers that specifically bind to PDGF-BB protein with K(d) < 3 nM within 3 rounds. Furthermore, we show that the aptamers identified by Quantitative Selection of Aptamers through Sequencing have approximately 3-8-fold higher affinity and approximately 2-4-fold higher specificity relative to those discovered through conventional cloning methods. Given that many biocombinatorial libraries are encoded with nucleic acids, we extrapolate that our method may be extended to other types of libraries for a range of molecular functions.

PNAS Lens Free Article Link: